Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA05/000522

International filing date: 06 April 2005 (06.04.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/559,452
Filing date: 06 April 2004 (06.04.2004)

Date of receipt at the International Bureau: 27 July 2005 (27.07.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PCT/CA 2005/000522

04 JULY 2005 04-07-05

PA 1333671

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

June 20, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/559,452*
FILING DATE: *April 06, 2004*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

N. Woodson

N. WOODSON
Certifying Officer

PTO/SB/16 (01-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Gerhard F.	Gries	Coquitlam, BC, Canada

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Pheromone for manipulating codling moth larvae

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number:

OR

☒ Firm or Individual Name	Gerhard Gries				
Address	484 Caribou Crescent				
Address					
City	Coquitlam	State	BC	Zip	V3C 4X7
Country	Canada	Telephone	604-944-4246	Fax	604-291-3496

ENCLOSED APPLICATION PARTS *(check all that apply)*

☐ Specification *Number of Pages* _____ ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☐ Other (specify) _____

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees.

☐ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): $ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

[Page 1 of 2]

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Gerhard Gries

TELEPHONE 604-944-4246

Date 22 March 04

REGISTRATION NO. _____ *(if appropriate)*

Docket Number: _____

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SB/16 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Docket Number	

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Zaid	Jumean	Sarnia, Ontario, Canada
Regine M.	Gries	Coquitlam, BC, Canada
Gerhard J.	Gries	Coquitlam, BC, Canada

Number _____ of _____

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Said Jumean
Examiner Name	
Art Unit	
Attorney Docket No.	

METHOD OF PAYMENT *(check all that apply)*

☒ Check ☐ Credit card ☒ Money Order ☐ Other ☐ None

☐ Deposit Account:

Deposit Account Number

Deposit Account Name

The Director is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☐ Credit any overpayments

☒ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				(Complete *(if applicable)*)	
Name (Print/Type)	Gerhard Gries	Registration No. (Attorney/Agent)		Telephone	604-944-4246
Signature				Date	22 March 04

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Dr. Gerhard Gries
484 Cariboo Crescent
Coquitlam, BC V3C 4X7
Canada

March 25, 2004

In the United States Patent and Trademark Office

US Patent and Trademark Office
2011 South Clark Place
Arlington, Virginia
22202 USA
Fax: (703) 746 4060

Mailed 2004

Sir:
Please file the enclosed Provisional Patent Application (PPA) papers listed below under 37 C.F.R. §. 1.53(b)(2).

Each of the undersigned understands:

A. This PPA is not a substitute for a Regular Patent Application (RPA), cannot be converted to an RPA, cannot get into interference with an RPA of another person, cannot be amended, will not be published, cannot claim any foreign priority, and will not mature into a patent;

B. If an RPA referring to this PPA is not filed within one year of the filing date of this PPA, this PPA will be worthless and will be destroyed;

C. Any desired foreign Convention application (including PCT applications) based upon this PPA must be filed within one year of the filing date of this PPA;

D. This PPA must contain written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact term as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention, 35 U.S.C. §. 112, ¶ 1. otherwise this PPA will be worthless.

E. Any RPA will be entitled to claim the benefit of the PPA only if such RPA names at least one inventor of this PPA and this PPA discloses such inventor's invention, as claimed in at least one claim of the RPA, in the matter provided in Item D above.

Inventor # 1, Name: Zaid Jumean
Legal Residence: 1375 Lakeshore Road, Sarnia, ON N7S 2M3, Canada

Inventor # 2, Name: Regine Gries
Legal Residence: 484 Cariboo Crescent, Coquitlam, BC V3C 4X7, Canada

Inventor # 3, Name: Gerhard Gries
Legal Residence: 484 Cariboo Crescent, Coquitlam, BC V3C 4X7, Canada

Title: Pheromone for manipulating codling moth larvae

☒ Specification, Sheets: 20 ☒ Drawings, Sheets: 12

☒ Check (US money order) or Credit Card Payment (Use PTO 2038) for $80.00 for

☒ small entity

☒ Return Receipt Postcard Addressed to:

Inventor # 3

Very respectfully,

Signature of Inventor # 1	*Signature of Inventor # 2*	*Signature of Inventor # 3*
Zaid Jumean	Regine Gries	Gerhard Gries
Name of Inventor # 1	*Name of Inventor # 2*	*Name of Inventor # 3*
519-542-7174	604-944-4246	604-944-4246
Telephone # of Inventor # 1	*Telephone # of Inventor # 2*	*Telephone # of Inventor # 3*
1375 Lakeshore Road, Sarnia, ON N7S 2M3, Canada	484 Cariboo Crescent, Coquitlam, BC V3C 4X7 Canada	484 Cariboo Crescent Coquitlam, BC V3C 4X7 Canada
Address	*Address*	*Address*

March 25, 2004

Synthetic Aggregation Pheromone for Manipulating the Behaviour of Codling Moth, *Cydia pomonella,* Larvae

Zaid Jumean, Regine Gries, and Gerhard Gries

Field of the Invention

This invention relates to a composition and procedure for manipulating the behaviour of codling moth larvae, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae). In particular, this invention relates to the use of specific pheromone components for manipulating the behaviour of *C. pomonella* larvae.

Background of the Invention

Larvae of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae), feed on and cause damage to apple, pear, walnut and other fruit and nut crops (Pedigo 1999). In a typical apple orchard, if left untreated, *C. pomonella* larvae can infest up to 95% of the crop resulting in major economic loss (Pedigo 1999).

In temperate regions, larvae feed from June to August within (apple) fruits (Dolstad 1985). In August, larvae exit fruits and seek pupation sites, often on trunks of fruit-bearing trees. Spinning cocoons in which to pupate, larvae produce an aggregation pheromone that attracts or arrests other *C. pomonella* larvae (Duthie *et al.* 2003). Components of this aggregation pheromone also attract the parasitic wasp *Mastrus ridibundus* (Hymenoptera: Ichneumonidae) which parasitize *C. pomonella* prepupae inside cocoons (Jumean *et al.*, unpublished). Synthetic aggregation pheromone in trapping devices would allow behavioural manipulation of *C. pomonella* larvae.

There are several patents listed in the United States Patent and Trademark Office database under the keyword *Cydia pomonella*. Two patents are concerned with the

synthesis of attractants for *C. pomonella*, as follows: U.S. Pat. No. 3,943,157 "Synthesis of codling moth attractant" reports the synthesis of codling moth sex pheromone, and U.S. Pat. No. 5,599,848 "Preparation, intermediates for the preparation and use of a mixture of dodecadienol isomers" reports a process for preparing and using the mixture of 8*E*,10*E*-dodecadienol, 8*E*,10*Z*-dodecadienol, 8*Z*,10*E*-dodecadienol, and 8*Z*,10*Z*-dodecadienol for interference of mating of *C. pomonella* adults. Three additional patents are concerned with methods of interfering with mating of *C. pomonella* adults, as follows: U.S. Pat. No. 6,395,775 "Combating pest insects" reports the use of the sex pheromone *E*8,*E*10-dodecadien-1-ol in combination with one or more behavioural antagonists or behavioural synergists for *C. pomonella* control. U.S. Pat. No. 4,734,281 "Method for concurrently emitting vapours of sex pheromones of different insects" reports the use of sex pheromone dispensers for controlling the population of two or more species of insect pests in the field, including *C. pomonella*. Finally, U.S. Pat. No. 6,528,049 "Bisexual attractants, aggregants, and arrestants for adult and larvae of codling moth and other species of lepidoptera" reports a method for monitoring and control of *C. pomonella* using attractants and arrestants from pears or apples. All of the behaviour-modifying compounds claimed for control of *C. pomonella* in the patents referred to above are very different from the attractive pheromone components produced by *C. pomonella* larvae, claimed in this application for attraction or arrestment of *C. pomonella* larvae that forage for suitable pupation sites.

Summary of the Invention

We reveal pheromone components which attract or arrest male and female *C. pomonella* larvae. These pheromone components are derived from silk produced when either male of female larvae spin cocoons.

The essence of the invention is the preparation and implementation of these pheromone components for manipulating the behaviour of *C. pomonella* larvae. Pheromone components can be used in all possible combinations and ratios. Pheromone component compositions can be contained in slow release devices. Devices can be held in traps to retain male and female *C. pomonella* larvae. The invention can be used in combination with other tactics employed to control *C. pomonella* adults for protection of apple, pear, walnut, and other fruit and nut crops from *C. pomonella*.

The invention is directed to a composition of chemicals for manipulating the behaviour of *C. pomonella* larvae, said composition comprising pheromone components in all possible combinations and ratios selected from the group consisting of: 1) heptanal; 2) sulcatone; 3) myrcene; 4) octanal; 5) 3-carene; 6) (+)-limonene; 7) (*E*)-2-octenal; 8) nonanal; 9) (*E*)-2-nonenal; 10) decanal; and 11) geranylacetone.

The composition can be contained in, or released from, slow release devices. The composition can be contained in, or released from, a trap that captures attracted *C. pomonella* larvae.

The invention is also directed to an apparatus for attracting or arresting *C. pomonella* larvae, said apparatus containing a composition comprising pheromone components in all possible combinations and ratios selected from the group consisting of: 1) heptanal; 2) sulcatone; 3) myrcene; 4) octanal; 5) 3-carene; 6) (+)-limonene; 7) (*E*)-2-octenal; 8) nonanal; 9) (*E*)-2-nonenal; 10) decanal; and 11) geranylacetone.

The invention is also directed to a bait and apparatus for deployment in orchards susceptible to *C. pomonella*, said bait incorporating pheromone components in all possible combinations and ratios and an apparatus which is suitable for *C. pomonella* larvae to pupate in.

The invention also pertains to a method of manipulating the behaviour of *C. pomonella* larvae which comprises exposing the insects to one or more pheromone components according to the invention.

The invention also pertains to a method of diagnosing whether protection of an apple, pear, walnut or other fruit or nut crop is warranted, comprising exposing apple, pear, walnut, or other fruit or nut crops to a composition of two or more pheromone components according to the invention, and determining whether any *C. pomonella* larvae are attracted or arrested by the composition of pheromone components.

The invention includes a method of protecting apple, pear, walnut, or other fruit or nut crops from attack by *C. pomonella* larvae by deploying proximate to apple or pome fruit crops the composition of pheromone components according to the invention.

Drawings

Drawings illustrate specific embodiments of the invention, but should not be construed as restricting the spirit or scope of the invention in any way:

FIG. **1** illustrates graphical data of responses of male or female *Cydia pomonella* larvae to pitfall devices baited with 1-day-old *C. pomonella* cocoons containing a male or female *C. pomonella* larva/prepupa.

FIG. **2** illustrates flame ionization detector (FID) and electroantennographic detector (EAD, female *Mastrus ridibundus* antenna) responses to ten cocoon-spinning *Cydia pomonella* larvae hour equivalents of cocoon volatile extracts.

FIG. **3** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with natural or synthetic pheromone components from cocoon spinning *C. pomonella* larvae.

FIG. **4** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with a synthetic 11-component pheromone blend at various doses.

FIG. **5** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with synthetic pheromone blends lacking specific classes of pheromone components.

FIG. **6** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with synthetic pheromone blends lacking individual pheromone components or specific groups of pheromone components.

FIG. **7** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with various blends of synthetic pheromone components.

FIG. **8** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with a synthetic 11-component pheromone blend, with components at natural ratios or increased amounts (x 10) of specific components.

FIG. **9** illustrates graphical data of responses of *Cydia pomonella* larvae in on-tree experiments to corrugated cardboard bands baited with live *C. pomonella* larvae/prepupae or synthetic pheromone blends.

FIG. **10** illustrates graphical data of responses of *Cydia pomonella* larvae in on-tree experiments to corrugated cardboard bands baited with synthetic pheromone blends at various doses.

FIG. **11** illustrates graphical data of responses of *Cydia pomonella* larvae in on-tree experiments to corrugated cardboard bands baited with synthetic pheromone blends, with specific components increased (x 10).

FIG. **12** illustrates graphical data of responses of *Cydia pomonella* larvae in on-tree experiments to corrugated cardboard bands baited with synthetic pheromone blends, with components at natural ratios or increased amounts (x 10) of specific components.

Detailed Description of the Invention

Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

1. Response of male and female *Cydia pomonella* larvae to cocoon-spinning male or female larvae

Response of 5th instar *C. pomonella* larvae seeking pupation sites was tested in 2-choice Petri dish olfactometers (Duthie *et al.*, 2003), with modified Eppendorf tubes in pitfall devices preventing physical contact of larvae with test stimuli. For each of at least 30 replicates per experiment, one male or female larva was released in the center of the olfactometer, and its pupation site recorded 18-24 hours later. Test stimuli consisted of a

control corrugated cardboard (CB) strip (2.5 x 2.5 cm) or a treatment CB strip carrying five 1-day-old cocoons each containing either a male or female *C. pomonella* larva/prepupa.

Male and female *C. pomonella* larvae preferred to spin cocoons in pitfall devices baited with CB carrying five 1-day-old cocoons, with no preference for cocoons containing either male or female larvae/prepupae (FIG. 1).

FIG. 1 illustrates graphical data of responses of male or female *Cydia pomonella* larvae to pitfall devices baited with control corrugated cardboard (CB) strips or treatment CB strips carrying five 1-day-old cocoons containing either male or female *C. pomonella* larvae/prepupae. Asterisks on bars indicate a significant response to a particular treatment; Chi-square goodness of fit test with Yates' correction for continuity; * $P <$ 0.025; ** $P < 0.001$.

2. Acquisition, analyses, and bioassays of pheromone components produced by cocoon-spinning *Cydia pomonella* larvae

To capture airborne pheromone components from cocoon-spinning larvae, three-hundred 5th instar male and female larvae were placed in a cylindrical Pyrex glass chamber (15.5 x 20 cm). An empty chamber served as control. A water aspirator drew charcoal-filtered air at ~2 l/min through each chamber and through a glass column (140 x 1.3 mm OD) containing Porapak Q (50-80 mesh, Waters Associates, Inc., Milford, Massachusetts 01757). After 72 hours, the filters were desorbed with 3 ml of pentane and ether (95:5).

Extracts were concentrated under a nitrogen stream so that 1 μL was equivalent to ca. 10 cocoon-spinning larvae hour equivalents (10 CSLHE = volatiles released from 10 cocoon-spinning *C. pomonella* larvae during 1 hour).

Aliquots of 20 CSLHE of Porapak Q-captured volatiles were subjected to analysis by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn *et al.* 1975) using an antenna of *Mastrus ridibundus,* a specialist parasitic wasp of *C. pomonella* prepupae, as the electroantennographic detector. Using antennae from female *M. ridibundus*, instead of *C. pomonella* larvae, in these analyses was necessary because *C. pomonella* larvae have antennae too small for electrophysiolocial studies. It was also justified because host-seeking female *M. ridibundus* respond to the pheromone produced by cocoon-spinning *C. pomonella* larvae. In these GC-EAD analyses, 10 components (8 visible in FIG. 2) elicited responses from *M. ridibundus* antennae.

FIG. 2 illustrates flame ionization detector (FID) and electroantennographic detector (EAD: female *Mastrus ridibundus* antenna) responses to 10 CSLHE of Porapak Q extract. Chromatography: Hewlett Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m x 0.32 mm ID) coated with DB-23 (J & W Scientific, Folsom, California 95630, USA); linear flow velocity of carrier gas: 35cm/sec; injector and FID detector temperature: 240°C; temperature program: 1 min at 50°C, 10° C/min to 220°C. Full scan electron impact (EI) and chemical ionization (CI) mass spectra of EAD active compounds were obtained by GC-mass spectrometry (MS) using a Varian Saturn II Ion Trap GC-MS and a HP 5985B GC-MS, respectively, each fitted with the DB-210

or DB-5 column. Candidate pheromone components were identified as follows: 1. heptanal (0.85); 2. sulcatone (0.81); 3. myrcene (0.84); 4. octanal (0.94); 5. 3-carene (0.95); 6. (+)-limonene (13.00); 7. (*E*)-2-octenal (0.41); 8. nonanal (4.10); 9. (*E*)-2-nonenal (1.00); 10. decanal (1.40); 11. geranylacetone (0.50). Note: 1) 10 CSLHE = volatiles released from 10 cocoon-spinning larvae during 1 hour; 2) number in brackets refers to nanogram quantities present in 10 CSLHE; 3) (+)-limonene was not antenally active but was included in bioassay experiments as the most abundant component in extracts.

3. Response of *Cydia pomonella* larvae to blends of natural or synthetic candidate pheromone components in olfactometer experiments

In olfactometer experiments (following the general protocol as described above), *C. pomonella* larvae preferred Porapak Q volatile extract of cocoon-spinning *C. pomonella* larvae, and a synthetic blend (SB) of 11 candidate pheromone components, over a pentane control stimulus (FIG. 3).

FIG. 3 illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with Porapak Q volatile extract of cocoon-spinning larvae (180 CSLHE) or a synthetic blend (SB) of 11 candidate pheromone components. Asterisks on bars indicate a significant response to a particular treatment; Chi-square goodness of fit test with Yates' correction for continuity; $***P < 0.001$. Note: 1) 180 CSLHE = volatiles released from 180 cocoon-spinning *C. pomonella* larvae during 1 hour; 2) components in SB

consisted of: 1. heptanal; 2. sulcatone; 3. myrcene; 4. octanal; 5. 3-carene; 6. (+)-limonene; 7. (*E*)-2-octenal; 8. nonanal; 9. (*E*)-2-nonenal; 10. decanal; 11. geranylacetone.

Similar attractiveness of Porapak Q extract containing natural cocoon volatiles and the synthetic 11-component blend (SB) strongly suggested that all essential pheromone components were present in SB.

Of the 4 doses of SB (1, 10, 100, 1,000 CSLHE) bioassayed, 100 SB elicited the strongest response by *C. pomonella* larvae (FIG. 4).

FIG. 4 illustrates graphical data of responses of *Cydia pomonella* larvae in pitfall olfactometer experiments to 4 doses of a synthetic blend (SB) containing 11 candidate pheromone components. Bars with an asterisk indicate a significant preference for a particular treatment; Chi-square goodness of fit test with Yates' correction for continuity; ** $P < 0.005$. Note: components in SB consisted of: 1. heptanal; 2. sulcatone; 3. myrcene; 4. octanal; 5. 3-carene; 6. (+)-limonene; 7. (*E*)-2-octenal; 8. nonanal; 9. (*E*)-2-nonenal; 10. decanal; 11. geranylacetone.

To determine the critically important components in SB, individual or groups of pheromone components were deleted and such reduced blends bioassayed in olfactometer experiments (following the protocol described above).

SB lacking monoterpenes [(+)-limonene, myrcene, 3-carene] or ketones (sulcatone, geranylacetone) still elicited responses from *C. pomonella* larvae, whereas SB lacking aldehydes [heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-nonenal] was completely unattractive (FIG. **5**).

FIG. **5** illustrates graphical data of responses of *Cydia pomonella* larvae to pitfall devices baited with synthetic blends (SB) lacking either ketones [sulcatone, geranylacetone], monoterpenes [(+)-limonene, myrcene, 3-carene], or aldehydes [heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-nonenal]. Asterisks on bars indicate a significant preference for a particular treatment; Chi-square goodness of fit test with Yates' correction for continuity; * $P < 0.05$; ** $P < 0.01$.

SBs lacking saturated aldehydes [heptanal, octanal, nonanal, decanal] still elicited significant responses from *C. pomonella* larvae, whereas SBs lacking either unsaturated aldehydes [(*E*)-2-octenal, (*E*)-2-nonenal], sulcatone, or geranylacetone were as ineffective as pentane controls in eliciting response from *C. pomonella* larvae (FIG. **6**).

FIG. **6** illustrates graphical data of responses of *Cydia pomonella* larvae to synthetic blends (SB) lacking either saturated aldehydes [heptanal, octanal, nonanal, decanal], unsaturated aldehydes [(*E*)-2-octenal, (*E*)-2-nonenal], or individual components. The asterisk on bars indicates a significant preference for a particular treatment; Chi-square goodness of fit test with Yates' correction for continuity; * $P < 0.01$.

A rudimentary synthetic blend (RSB), comprising (*E*)-2-octenal, (*E*)-2-nonenal, sulcatone and geranylacetone, did not elicit a behavioural response by *C. pomonella* larvae. However, RSB in combination with either the monoterpene 3-carene, saturated aldehydes (octanal, nonanal, decanal), or 3-carene plus said aldehydes, induced significant attraction/arrestment by *C. pomonella* larvae (FIG. **7**).

FIG. **7** illustrates graphical data of responses of *Cydia pomonella* larvae to a 4-component rudimentary synthetic blend (RSB) [(*E*)-2-octenal, (*E*)-2-nonenal, sulcatone, geranylacetone] alone or in combination with either 3-carene, saturated aldehydes [octanal, nonanal, decanal], or both. Asterisks on bars indicate a significant preference for a particular treatment; Chi-square goodness of fit test with Yates' correction for continuity; * $P < 0.01$; ** $P < 0.005$.

The 11-component synthetic blend (SB), when tested at the low dose of 10 cocoon-spinning larval hour equivalents and at natural compound ratios (Fig. **2**), did not induce behavioural responses by *C. pomonella* larvae. However, a 10-fold increase of both (*E*)-2-octenal and (*E*)-2-nonenal, but not of either component singly, in SB resulted in significant attraction/arrestment of *C. pomonella* larvae (FIG. **8**).

FIG. **8** illustrates graphical data of responses by *Cydia pomonella* larvae to a synthetic blend (SB) of 11 components at natural ratios (Fig. **2**), or at a 10-fold increase of either or both (*E*)-2-octenal and (*E*)-2-nonenal. The asterisk indicates a significant preference for a particular treatment; Chi-square goodness of fit test with Yates' correction for

continuity; * $P < 0.05$. Note: SB was tested at 10 cocoon-spinning larval hour equivalents.

4. On-tree testing of natural or synthetic larval aggregation pheromone

In on-tree experiments, maple (*Acer* spp.) trees (10-16 cm diameter at a height of 45 cm) were banded with corrugated cardboard strips (5 cm wide) 45 cm above ground. Strips were divided into two halves, with test stimuli applied to the waxed, center portion of each half. For each replicate in all experiments, twenty 5th instar *C. pomonella* larvae were released from a thin, circular platform affixed to the base of the tree's main branch crotch (~1.50 m above ground). Experiments were initiated after dusk, and numbers of *C. pomonella* larvae cocooning in treatment or control halves of corrugated cardboard strips recorded 10-12 hours later.

EXAMPLE # 1

In experiment 31, significantly more *C. pomonella* larvae cocooned in treatment halves of corrugated cardboard strips, bearing twenty-five 1-day-old *C. pomonella* cocoons with female larvae/prepupae inside, than in unbaited control halves. Similarly, in experiment 32 significantly more *C. pomonella* larvae cocooned in halves of corrugated cardboard strips baited with a synthetic blend (SB) of pheromone components than in halves treated with a solvent control (FIG. **9**).

FIG. **9** illustrates graphical data of responses by *Cydia pomonella* larvae in on-tree experiments 31 (12 replicates) and 32 (18 replicates) to corrugated cardboard (CB) strips.

Treatment halves of CB strips carried twenty-five 1-day-old cocoons containing female *C. pomonella* larvae/prepupae (Exp. 31), or were baited with a synthetic blend (SB) of 11 components at 1,000 cocoon-spinning larval hour equivalents (Exp. 32). Control halves were bare (Exp. 31), or were impregnated with the equivalent amounts of solvent (Exp. 32). Asterisks indicate a significant preference for a particular stimulus; Wilcoxon paired-sample test; * $P < 0.01$; ** $P < 0.005$.

EXAMPLE # 2

In on-tree experiments 33-35, the synthetic blend (SB) at 1,000 cocoon-spinning larvae hour equivalents (CSLHE), but not at 100 or 10,000 CSLHE, attracted/arrested significantly more *C. pomonella* larvae than did a solvent control stimulus (FIG. 10)

FIG. 10 illustrates graphical data of responses by *Cydia pomonella* larvae in on-tree experiments 33 (18 replicates), 34 (18 replicates) and 36 (12 replicates) to corrugated cardboard (CB) strips impregnated with a synthetic blend (SB) of 11 components at 100, 1,000, or 10,000 cocoon spinning larvae hour equivalents, or a solvent control. The asterisk indicates a significant preference for a particular stimulus; Wilcoxon paired-sample test; * $P < 0.01$.

EXAMPLE # 3

In on-tree experiment 36, significantly more *C. pomonella* larvae cocooned in halves of corrugated cardboard (CB) strips impregnated with a synthetic blend (SB) of 11 components, tested at 100 cocoon-spinning larval hour equivalents with increased

amounts (x 10) of (*E*)-2-octenal and (*E*)-2-nonenal, than on halves impregnated with a solvent control (FIG. **11**).

FIG. **11** illustrates graphical data of responses by *Cydia pomonella* larvae in on-tree experiment 36 (12 replicates) to corrugated cardboard (CB) strips impregnated with a synthetic blend (SB) of pheromone components or a solvent control. The asterisk indicates a significant preference for a particular stimulus; Wilcoxon paired-sample test; * $P < 0.02$. Note: SB was tested at 100 cocoon-spinning larval hour equivalents with increased amounts (x 10) of (*E*)-2-octenal and (*E*)-2-nonenal.

EXAMPLE # 4

In concurrently run on-tree experiments 37 and 38, significantly more *C. pomonella* larvae cocooned in halves of corrugated cardboard strips impregnated with a synthetic blend (SB) of 11 components at doses of 1,000 cocoon-spinning larval hour equivalents (CSLHE), or a modified synthetic blend at 100 CSLHE, than in halves impregnated with the equivalent amount of a solvent (pentane) control (FIG. **12**).

FIG. **12** illustrates graphical data of responses by *Cydia pomonella* larvae in concurrently run on-tree experiments 37 (24 replicates) and 38 (24 replicates) to halves of corrugated cardboard (CB) strips impregnated with synthetic blends (SB) of 11 components. An asterisk indicates a significant preference for a particular stimulus; Wilcoxon paired-sample test; * $P < 0.005$. Note: In experiment 38, the SB at 100 cocoon-spinning larvae hour equivalents contained increased amounts (x 10) of (*E*)-2-octenal and (*E*)-2-nonenal.

References

U.S. PATENT DOCUMENTS

Henrick, C. A. and Siddall, J. B. 3/1976. Synthesis of codling moth attractant. U.S. Pat. No. 3,943,157.

Klein, U., Neumann, U., Mackenroth, W., Renz, G., Krieg, W., Mackenroth, C., Buschman, E., DeKramer, J. J., and Milli, R. 2/1997. Preparation, intermediates for the preparation and the use of a mixture of dodecadienol isomers. U.S. Pat. No. 5,599,848.

Light, D. M., Henrick, C. A. 3/2003. Bisexual attractants, aggregants, and arrestants for adults and larvae of codling moth and other species of lepidoptera. U.S. Pat. No. 6,528,049.

Lofqvist, J., Bengtsson, M., and Witzgall, P. 5/2002. Combating pest insects. U.S. Pat. No. 6,395,775.

Yamamoto, A., Ogawa, K., and Nagura, S. 3/1988. Method for concurrently emitting vapors of sex pheromones of different insects. U.S. Pat. No. 4,734,281.

OTHER PUBLICATIONS

Arn, H., Städler, E., and Rauscher, S. 1975. The electroantennographic detector – a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Zeitschrift fur Naturforschung* 30: 722-25.

Dolstad, K. D. 1985. Biology and control of the codling moth in the Pacific Northwest. M.P.M. Professional Paper. Simon Fraser University, Burnaby.

Duthie, B., Gries, G., Gries, R., Krupke, C. and Derksen, S. 2003. Does pheromone based aggregation of codling moth larvae help procure future mates? *Journal of Chemical Ecology* 29: 425-436.

Pedigo, L. P. 1999. Entomology and pest management. Prentice-Hall, Inc., New Jersey.

WHAT IS CLAIMED IS:

1. A composition of chemicals for manipulating the behaviour of *Cydia pomonella* larvae, said composition comprising two or more chemicals in all possible combinations and ratios selected from the group consisting of: 1) heptanal; 2) sulcatone; 3) myrcene; 4) octanal; 5) 3-carene; 6) (+)-limonene; 7) (*E*)-2-octenal; 8) nonanal; 9) (*E*)-2-nonenal; 10) decanal; 11) geranylacetone.

2. A composition as claimed in claim **1** wherein the composition is contained in, or released from, slow release devices.

3. A composition as claimed in claim **1** wherein the composition is contained in, and released from, an apparatus that provides suitable pupation sites for attracted *Cydia pomonella* larvae.

4. An apparatus for attracting *Cydia pomonella* larvae, said apparatus containing a composition comprising two or more chemicals in all possible combinations and ratios selected from the group consisting of: 1) heptanal; 2) sulcatone; 3) myrcene; 4) octanal; 5) 3-carene; 6) (+)-limonene; 7) (*E*)-2-octenal; 8) nonanal; 9) (*E*)-2-nonenal; 10) decanal; 11) geranylacetone.

5. An apparatus as claimed in claim **4** wherein the apparatus contains an insect-killing agent.

6. A bait and apparatus for deployment in an area containing fruit or nut crops, said bait incorporating a composition of chemicals as claimed in claim **1**, and said apparatus providing suitable pupation sites for *Cydia pomonella* larvae.

7. A method of manipulating the behaviour of *Cydia pomonella* larvae which comprises exposing larvae to two or more chemicals as claimed in claim **1.**

8. A method of diagnosing whether protection of apple, pear, walnut, or other fruit or nut crops is warranted, comprising exposing the fruit or nut crop to a composition as claimed in claim **1,** and determining whether *Cydia pomonella* larvae are attracted by the composition.

9. A method of protecting apple, pear, walnut, or other fruit or nut crops from attack by *Cydia pomonella* larvae by deploying proximate to fruit or nut crops a composition as claimed in claim **1.**

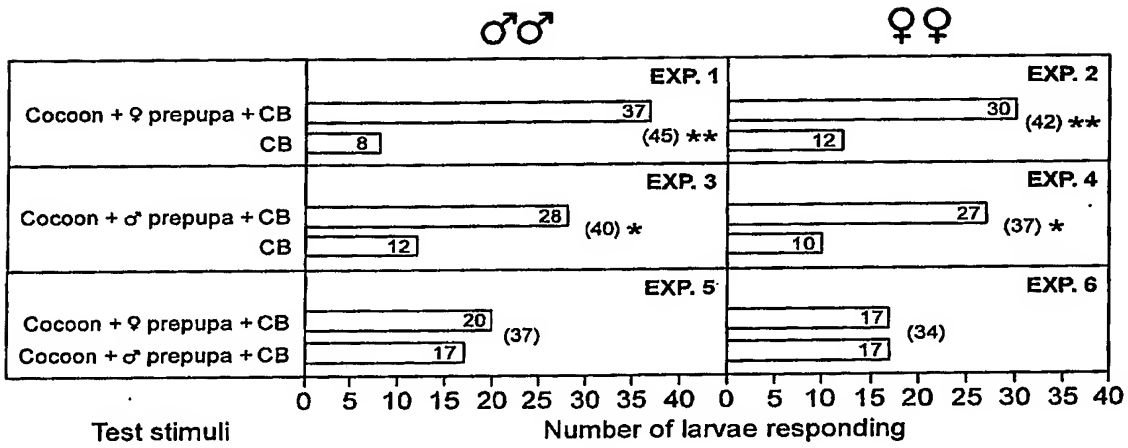

FIG. 1

Pomonella fig 1

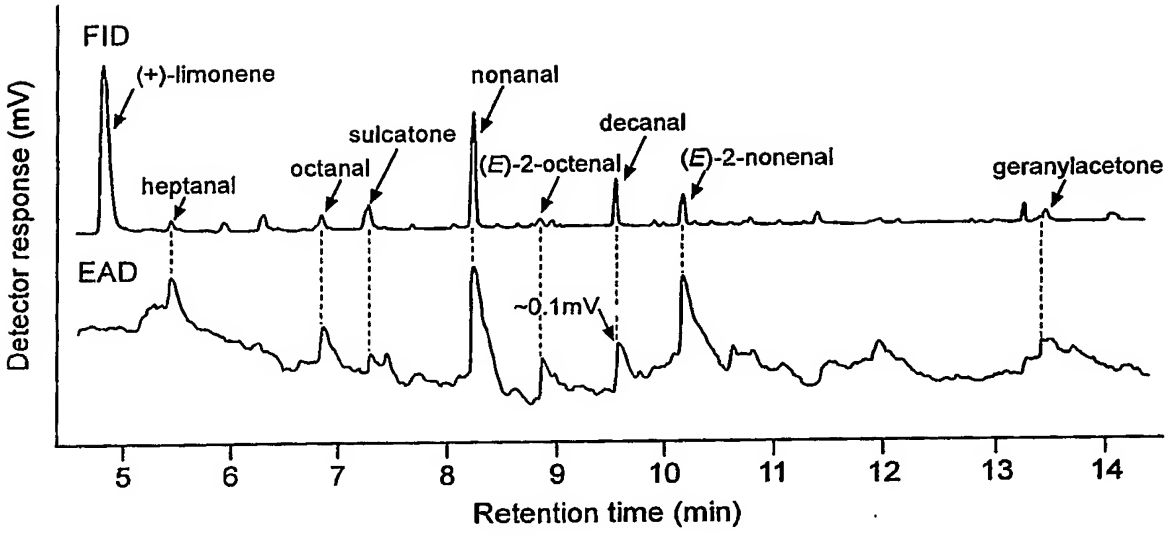

FIG. 2

Pomonella-ridi-fig 2

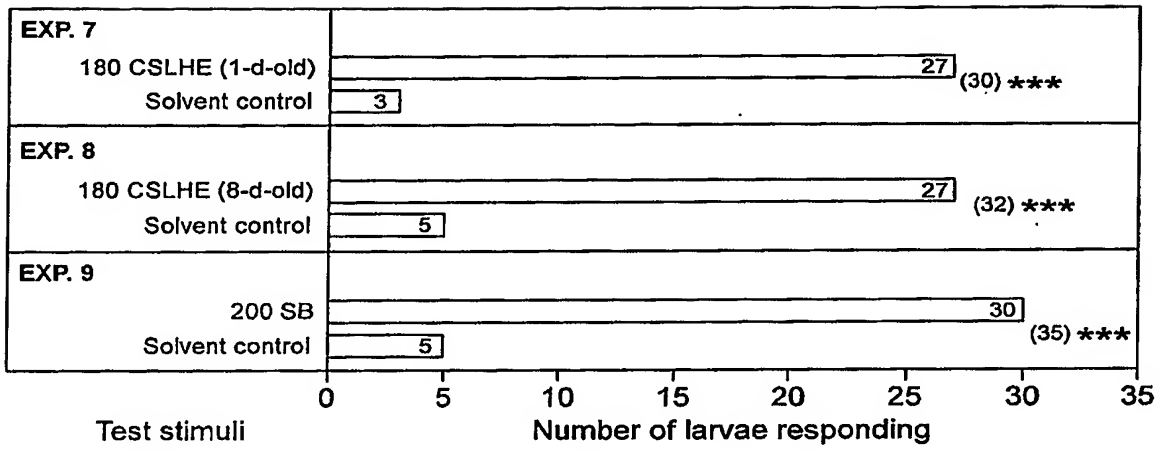

FIG. 3

Pomonella fig 3

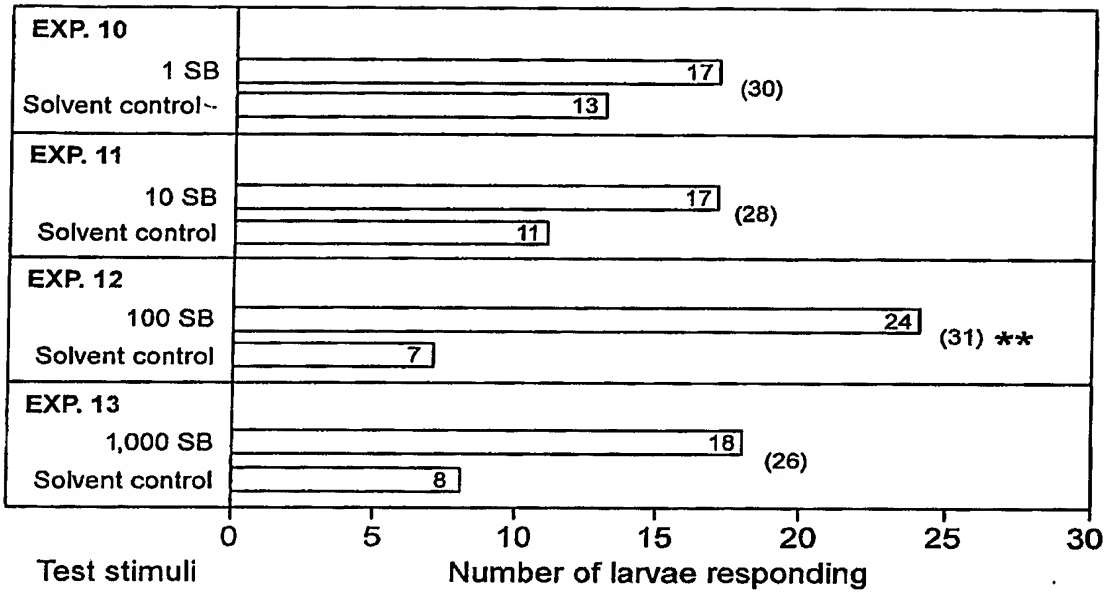

FIG. 4

Pomonella fig 4

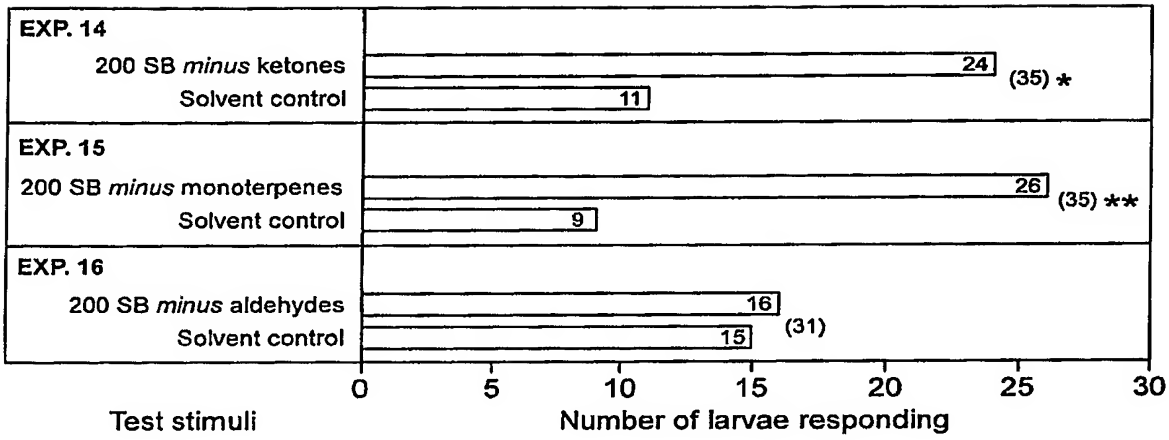

FIG. 5

Pomonella fig 5

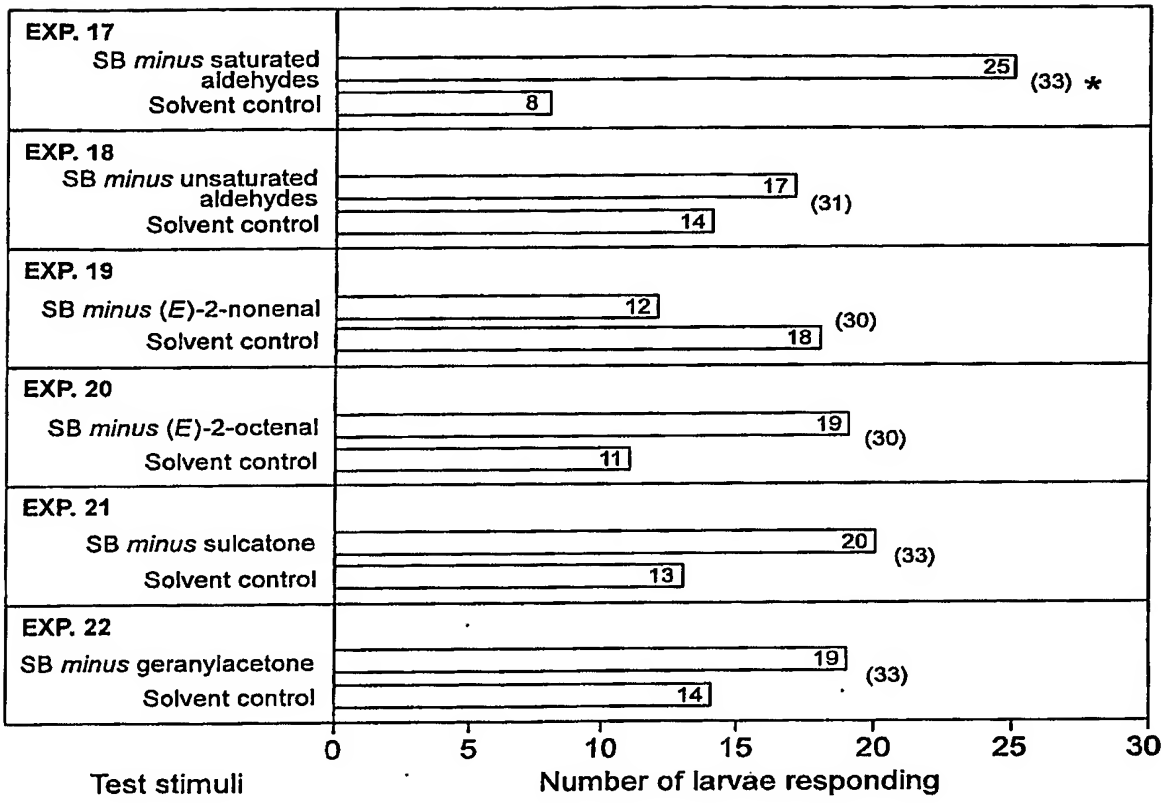

FIG. 6

Pomonella fig 6

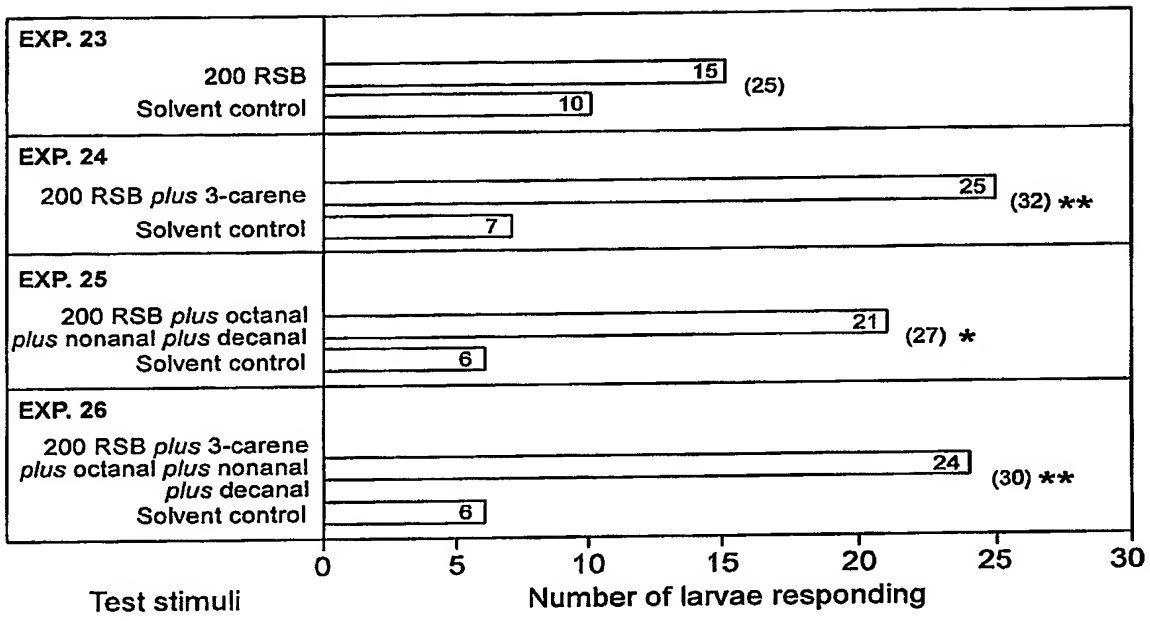

FIG. 7

Pomonella fig 7

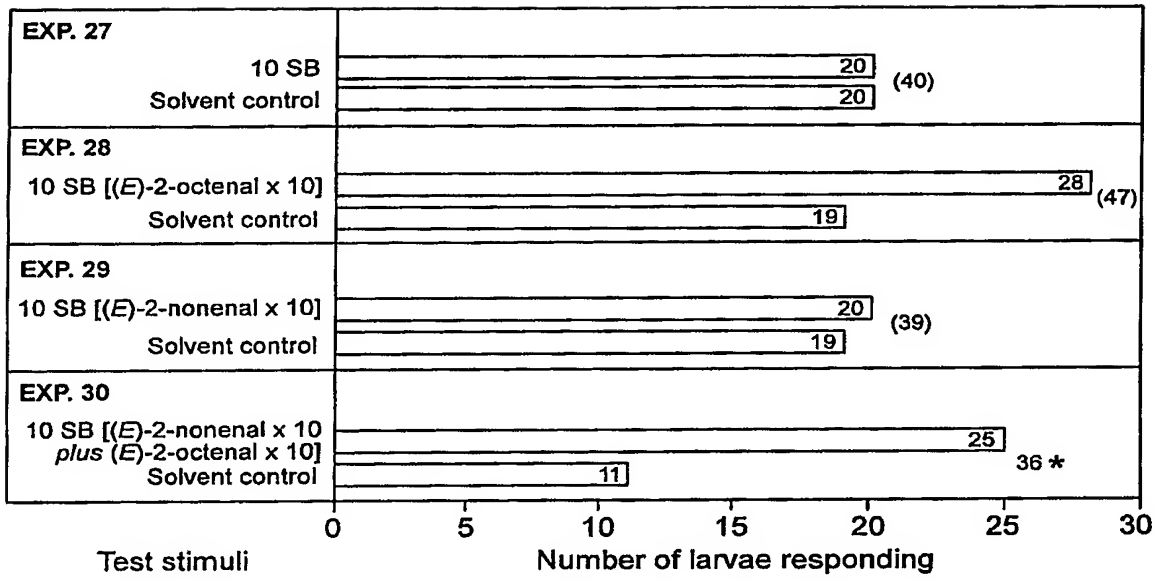

FIG. 8

Pomonella fig 8

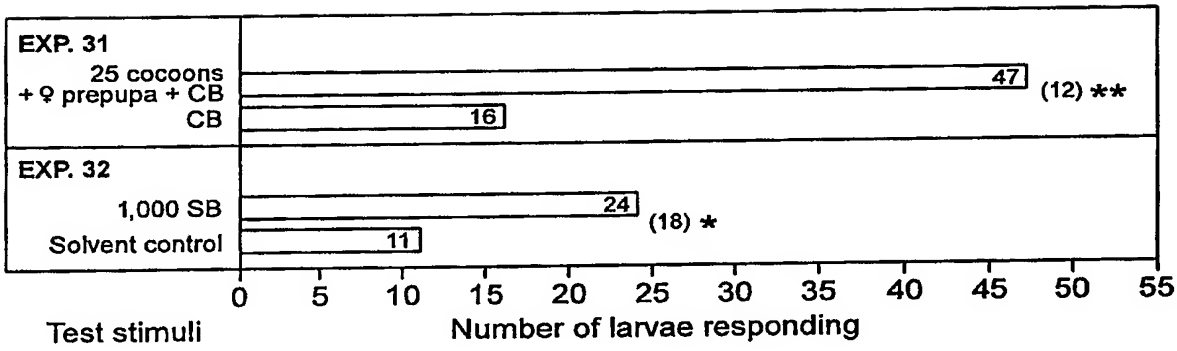

FIG. 9

Pomonella fig 9

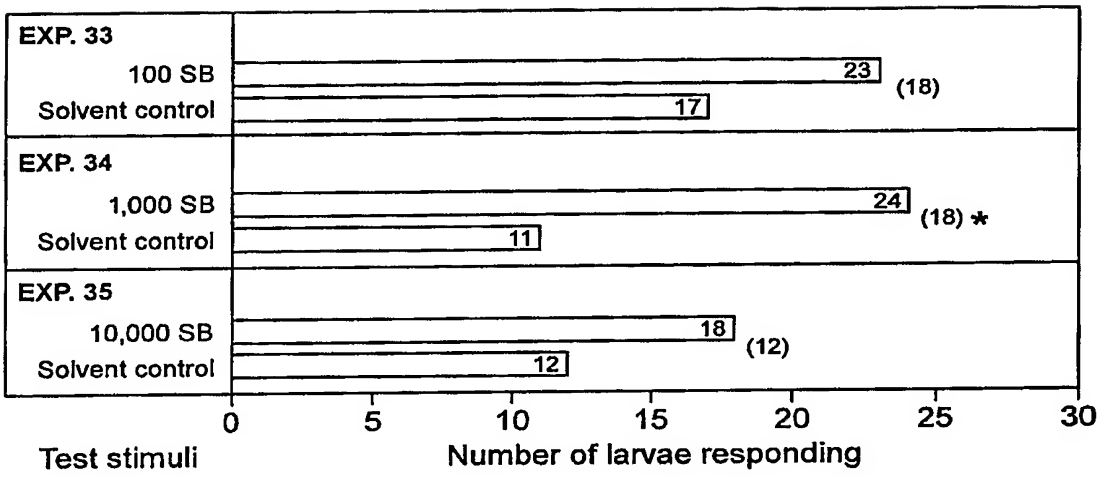

FIG. 10

Pomonella fig 10

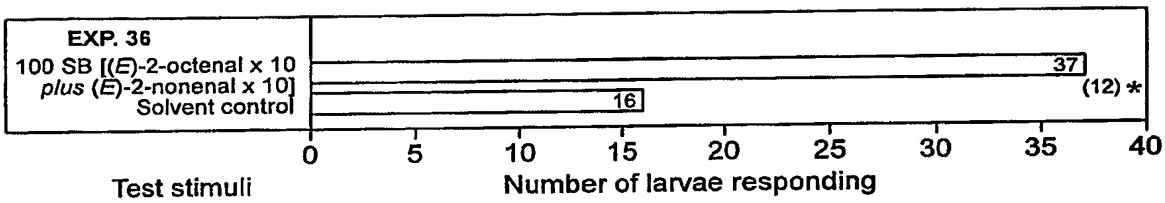

FIG. 11

Pomonella fig 11

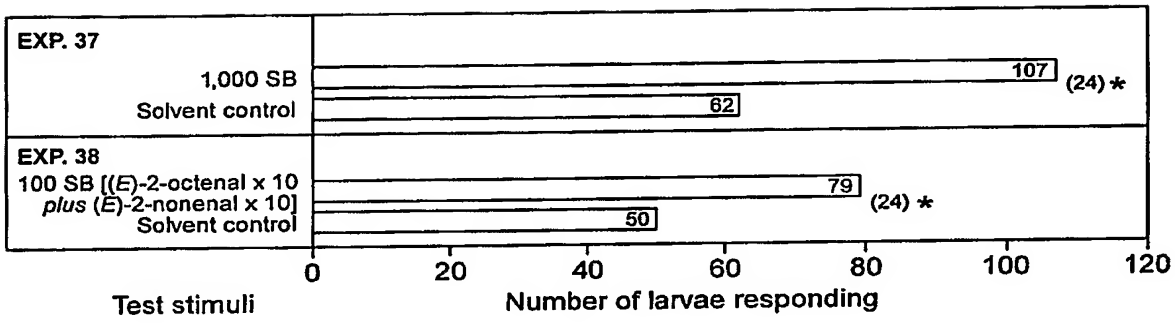

FIG. 12

Pomonella fig 12